Identification of predictive biomarkers for response of R/R DLBCL patients treated with loncastuximab tesirine using low-pass whole-genome cell-free DNA sequencing (cfDNA-WGS)

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INTRODUCTION AND OBJECTIVES

- Plasma profiling can non-invasively identify biomarkers associated with treatment outcomes and reveal mechanisms underlying drug resistance.
- Loncastuximab tesirine (lonca), an antibody-drug conjugate comprising a humanized anti-CD19 antibody and a pyrrolobenzodiazepine dimer cytotoxin, is indicated for relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) after ≥2 systemic treatments.
- The objective of this study was to investigate biological signatures associated with response to lonca using plasma-derived cell-free DNA (cfDNA) samples from 145 R/R DLBCL patients in a phase 2 trial (NCT3589469, LOTIS-2).

METHODS

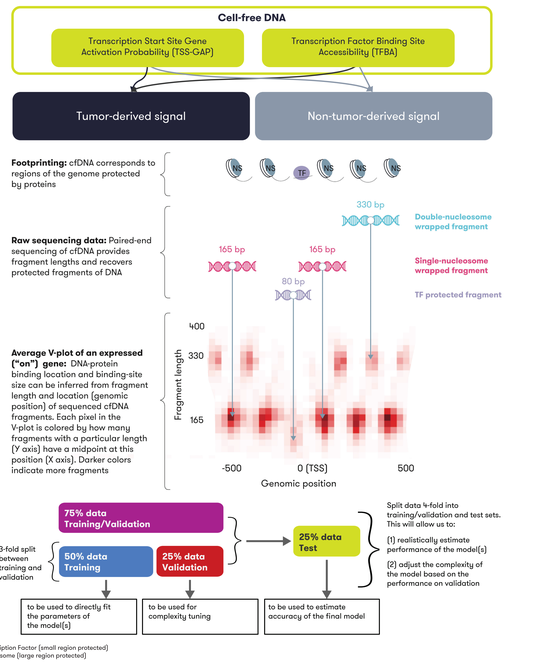
- Cell-free DNA (cfDNA) was extracted from 100 patients at baseline and 80 patients on treatment (cycle 2 day 1; C2D1).
- Low-pass whole-genome sequencing was performed to characterize cfDNA fragments, which reflect nucleosome protection and chromatin state.
- Gene activation for protein-coding genes was inferred from fragment distribution around transcription start sites[1] to generate Transcription Start Site Gene Activation Probability scores (TSS-GAP; a schematic of the approach is shown in **Figure 1**).
- Binding activity of 504 cancer-associated transcription factors (TFs) was estimated by inferring the level of chromatin accessibility from ~1000 binding sites per factor across the genome to give Transcription Factor Binding Association (TFBA) scores[2].
- Class comparison statistics for TSS-GAP and TFBA features were performed using the Wilcoxon signed rank test for paired longitudinal comparisons.
- Gene Set Enrichment Analysis (GSEA) was performed using Molecular Signatures Database (MSigDB) sets. P-values were corrected for multiple hypothesis testing to control the False Discovery Rate (FDR).
- A multivariate classifier for response assessment according to 2014 Lugano classification was constructed by applying L1-regularized logistic regression on both TSS-GAP and TFBA features (as in **Figure 1**).
- Clinical lab data including Chem-20 panel results, complete blood count results, and additional immunophenotyping (CD5/19/20/21/23/43, BCL1/2/6, Cyclin D1, ZAP-70) were incorporated into the multivariate modeling by concatenating these values with the TSS-GAP scores.

Table 1. Cohort description and profiling

	CR/PR	SD/PD	p-value
Age	63 (23-83)	63 (24-82)	0.96[a]
Sex			
Male	31 (70.5%)	26 (63.4%)	0.65[b]
Female	13 (29.5%)	15 (36.6%)	0.65[b]
Prior Therapies			
Rituximab-CHOP	25 (61%)	32 (72.7%)	0.97[b]
Rituximab-GDP	6 (14.6%)	8 (18.2%)	0.97[b]
Rituximab-ICE	13 (31.7%)	15 (34.1%)	0.97[b]

[a]1-way ANOVA
[b]Chi-square frequency test

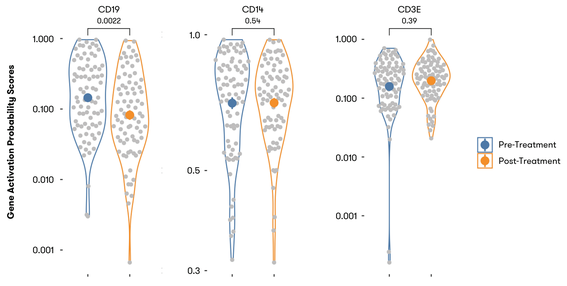
Figure 1. Cohort description and profiling



TF = Transcription Factor (small region protected)
NS = Nucleosome (large region protected)

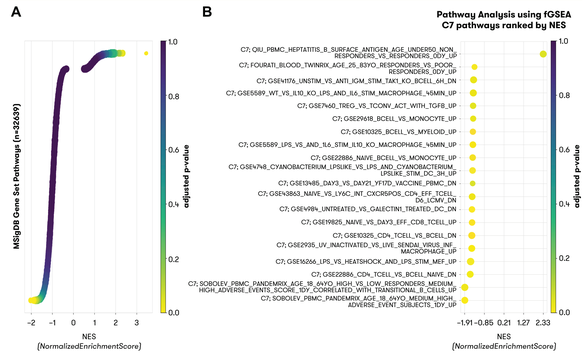
- Regression modeling strategy including training/validation folds
- Logistic regression using L1 regularization

Figure 2. Lonca treatment reduces cfDNA-estimated levels of CD19 but not of other immune markers



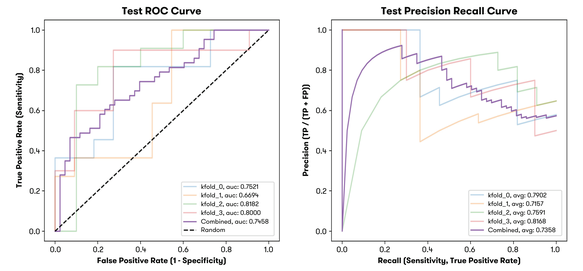
- We evaluated longitudinal changes in TSS-GAP scores occurring during treatment with lonca between C2D1 and baseline
- Estimated levels of CD19, a proxy for B-cell abundance, significantly decreased after treatment (Wilcoxon signed-rank paired test: p = 2.2E-3), which supports the hypothesis that lonca is actively reducing B-cells
- Markers of two other immune populations showed no significant change (CD14, p = 0.54; CD3E, p = 0.39).

Figure 3. Gene set enrichment analysis reveals significantly decreased signatures for B-cell gene sets in treated cases compared to baseline



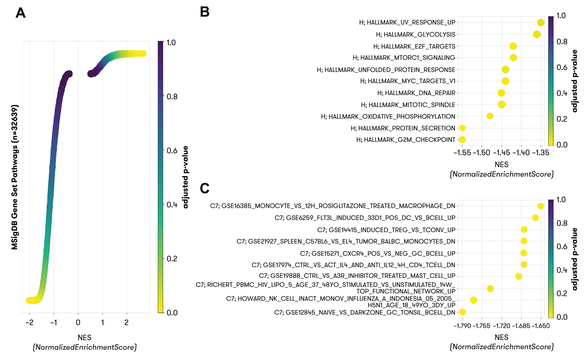
- GSEA identified 157 significantly enriched sets (152 reduced after treatment; False Discovery Rate (FDR) < 5%), including B-cell gene set signatures decreasing from baseline
- The 20 significantly enriched immunologic signatures (C7) gene sets (FDR < 5%) reveal downregulation of B-cell-related sets amongst other signatures of immune suppression compared to baseline.

Figure 4. Multivariate classifier distinguishes responders from non-responders at baseline in cross-validation



- To construct a multivariate classifier for response using baseline data, we applied a L1-logistic regression model to both the computational TFBA and TSS-GAP features to identify the best features.
- Early results with TFBA scores suggested transcription factor features to be the most informative (AUC = 0.65), so we selected the 2,751 TSS-GAP scores for all transcription factors across the genome to include in the multivariate classifier.
- Clinical lab data, from standard blood chemistry tests and immunophenotyping as detailed in the methods, was also incorporated into the modeling by concatenating it with the TSS-GAP TF scores.
- Shown here is the final model combining TSS-GAP TF scores and clinical lab data to distinguish patients based on predicted response groups in cross-validation (AUC = 0.75; AUPRC = 0.74).

Figure 5. Gene set enrichment analysis reveals higher activation levels of proliferative and immune gene sets in non-responders at baseline



- GSEA of TSS-GAP scores between responders vs non-responders revealed 3145 significant gene sets (FDR<5%) out of 32639 tested.
- 11 significant Hallmark (H) gene set signatures (FDR<5%) reveal higher activation levels of proliferative activity sets in non-responders at baseline.
- 1257 significant immunologic signatures (C7) gene set signatures (FDR<5%) reveal changes in immune activation; notably evidence of increased immune activity is seen in non-responders relative to responders at baseline. Top 10 gene sets ranked by score are plotted for visualization.

CONCLUSIONS

- Using our platform, we characterized a cohort of R/R DLBCL patients at baseline and on treatment (cycle 2 day 1; C2D1) with loncastuximab tesirine.
- The results showcase the potential of our approach to identify markers associated with response to lonca and suggest mechanisms of resistance, potentially informing rational drug combination research.
- Our modeling efforts integrating TSS-GAP and clinical data indicate the potential of this approach to predict lonca efficacy, pending additional validation.
- Future studies with our platform may enable targeted precision medicine applications and therapeutic decisions.

REFERENCES

1. Saint John J *et al* AACR 2019
2. Ulz P *et al Nature Communications* 2019

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